भारतीय मानक
Indian Standard

IS 1479 (Part 1) : 2016

(Reaffirmed 2021)

डेरी उद्योग के लिए परीक्षण के तरीके

भाग 1 दुग्ध की तेजी से परीक्षा

(पहला पुनरीक्षण)

Methods of Test for Dairy Industry

Part 1 Rapid Examination of Milk

(First Revision)

ICS 67.100.10

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भारतीय मानक ब्यूरो
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Dairy Products and Equipment Sectional Committee, FAD 19

FOREWORD

This Indian Standard (Part 1) (First Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Dairy Products and Equipment Sectional Committee had been approved by the Food and Agriculture Division Council.

Milk is one of the most important commodities entering trade and required in every day life as an article of food. The basic public health and economic considerations require that consumers should be provided with pure milk, free from pathogenic bacteria. To maintain quality standards, control operations have to be performed at all stages of the production of milk which include maintenance of sanitary conditions of byres; cleanliness of utensils and of the milking machines, if used; and care during storage and handling. Wilful adulteration at the producing or supplying centre, by carriers or by middlemen, has to be prevented. The only means of ensuring quality, when the purchaser cannot exercise a direct control over the production, transport and handling of milk, is by subjecting representative samples to chemical and bacteriological analysis. The need for having uniform methods for assessing the quality of milk at the time of purchase or sale is, therefore, obvious.

It may not be often necessary nor is it possible to subject all the samples of milk to a regular and elaborate chemical and bacteriological analysis. At milk collecting centres, rapid examination of milk has to be carried out for routine check with the object of ensuring purity of milk, its hygienic quality and fitness for acceptance as raw milk or for further processing. In order to provide uniform methods for this purpose, such of the methods as can be rapidly employed have been compiled as Part 1 of this standard.

Besides performing rapid tests, it is often necessary to carry out accurate determinations of various constituents for specific purposes. Methods for these chemical tests are covered in Part 2 of this standard [IS 1479 (Part 2) : 1961 'Methods of test for dairy industry: Part 2 Chemical analysis of milk'], while elaborate methods for bacteriological examination are covered in Part 3 [IS 1479 (Part 3) : 1977 'Methods of test for dairy industry: Part 3 Bacteriological analysis of milk (*first revision*)'].

Apart from the above the following additional Parts have also been published:

IS 1479 (Part 4) : 2009	Methods of test for dairy industry: Part 4 Determination of freezing point of milk— Thermistor cryoscopoe method (reference method)
IS 1479 (Part 5) : 1962	Methods of test for dairy industry: Part 5 Methods of dairy plant control

This standard was first published in 1960. This revision has been undertaken to incorporate the following modifications:

a) In the clause 'Detection of Adulterants', new tests, namely Holde's test for detection of mineral oil, determination of maltodextrins in milk, detection of cane sugar, detection of glucose, detection of vegetable oils/foreign fat in milk, test for soluble sulphates and test for detection of soya powder in milk have been included;

b) In the clause 'Detection of Preservatives', existing turmeric paper test for boric acid has been replaced with glycerol test and new test for hydrogen peroxide has been included;

c) In the clause 'Detection of Neutralizers', Rosalic acid test for carbonates has been revised and new tests, namely before and after boiling acidity test for neutralizer (NaOH) and test for sodium ions have been included;

d) New direct microscopic somatic cell count has been incorporated for detection of mastitis;

e) Alizarin-Alcohol test, ten minute resazurin test and test for alkalinity of ash have been deleted as these are no longer in use; and

f) Reference to Indian Standards have also been updated.

In reporting the result of a test or analysis made in accordance with this standard, is to be rounded off, it shall be done in accordance with IS 2 : 1960 'Rules for rounding off numerical values *(revised)*'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Indian Standard

METHODS OF TEST FOR DAIRY INDUSTRY

PART 1 RAPID EXAMINATION OF MILK

(First Revision)

1 SCOPE

1.1 This standard (Part 1) specifies rapid methods that are normally required for assessing the quality of raw milk supplies intended for processing and manufacture. The specific test(s) to be employed would depend upon the purpose of the analysis.

1.2 This standard does not include such methods of chemical and bacteriological analysis as are used for the detailed examination of milk.

2 REFERENCES

The following Indian Standards contain provisions which through reference in this text, constitute provisions of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below :

IS No.	*Title*
323 : 2009	Rectified spirit for industrial use — Specification (*second revision*)
460 (Part 1) : 1985	Specification for test sieves: Part 1 Wire cloth test sieves (*third revision*)
1070 : 1992	Reagent grade water — Specification (*third revision*)
1224 (Part 1) : 1977	Determination of milk fat by the gerber method: Part 1 Milk (*first revision*)
1479 (Part 4) : 2009	Methods of test for dairy industry: Part 4 Determination of freezing point of milk — Thermistor cryoscopoe method (reference method) (*second revision*)
3508 : 1966	Method of sampling and test for *Ghee*
10083 : 1982	Method of test for determination of SNF (solids not fat) in milk by the use of lactometers
16326 : 2015	*Ghee* — Specification

3 QUALITY OF REAGENTS

Unless otherwise specified, pure chemicals and distilled water (*see* IS 1070) shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the experimental results.

4 SAMPLING OF MILK

4.0 General

Sampling of milk shall be done by an experienced person. It is not possible to lay down a single sampling procedure for milk which will be applicable in all cases. The method of sampling will vary according to the purpose for which the sample is collected and the tests which are to be carried out. Proper sampling, however, requires the most careful attention to the recommended procedures in this standard.

4.0.1 Samples may be required for chemical or bacteriological examination. All precautions shall be taken to prevent contamination and adulteration.

4.0.2 For chemical examination, the sampling equipment shall be clean and dry.

4.0.3 Samples for bacteriological examination shall be collected by a person trained in the technique of sampling for bacteriological work.

4.0.3.1 For bacteriological purposes, all equipment including plungers, sample bottles, rubber stoppers, shall be sterile and the samples shall be collected under aseptic conditions. Equipment shall be sterilized by one of the following methods:

a) Heating in a hot air oven for not less than 2 hours at 160°C, or
b) Autoclaving for not less than 15 min at 120°C.

NOTES

1 Under field conditions, equipment may be sterilized by immersion for at least 5 minutes in boiling water. Equipment treated by this method shall be used immediately.

2 Rubber stoppers shall be sterilized in an autoclave as in (b). Treatment by immersion in boiling water for not less than 10 min would be satisfactory if they are used immediately.

4.0.4 The sample collected for chemical analysis should be representative of the entire batch of milk that is being sampled. Since milk fat is of lower density than the other constituents of milk, it tends to rise to the surface. Thorough mixing of milk with a proper instrument which will reach the entire depth of the liquid is essential to ensure a representative sample of the entire batch. In small batches, it should be possible to accomplish mixing by pouring the entire quantity of milk from one container to another, three or four times. Larger batches of milk shall be thoroughly agitated by a hand stirrer, or by mechanical means. Milk churns easily at 26.5 to 29.5°C and agitation near this temperature shall be avoided.

4.1 Sampling from Individual Container

Pour the milk from one container to another, three or four times. Where this is not practicable, mix thoroughly with a plunger (*see* **4.6.1**). In mixing the milk, the plunger shall be allowed to fall to the bottom of the container and brought to the top of the milk as rapidly as possible not less than 10 times. The position of the plunger shall also be moved from place to place to ensure that the whole of the milk at the bottom of the vessel is thoroughly agitated and mixed with the upper layer. Any milk fat adhering to the neck and under the shoulder of the can shall be well mixed with the remainder of the milk. After thorough mixing, a sample shall be drawn immediately.

4.2 Sampling from Several Containers

The samples shall be taken after pouring the contents of the containers into a vat and mixing. When this is not possible, a composite sample is taken in the following manner from the containers after milk has been agitated and mixed. First, the milk shall be distributed as equally as possible among a number of containers. The cans shall not be filled, but the same quantity shall be placed in each. After mixing the contents of each can thoroughly, an equal volume of milk shall be taken from each. These portions shall be placed in another vessel, thoroughly mixed as described in **4.3** and a sample then taken.

4.2.1 Alternatively, where facilities exist for accurate measurements, a composite sample may be obtained by taking the same proportion of the milk therein from each container in a consignment after thorough mixing, collecting this in another vessel and taking a sample as described in **4.3**.

4.3 Sampling Bulk Units

When milk of uniform quality is supplied in bulk units (for example, cans filled from storage tanks), the number of random units to be sampled shall be as follows:

Total No. of Units	*No. of Units to be Selected*
1	1
2-5	2
6-20	3
21-60	4
61-100	5
Over 100	5 plus *one for each additional 100 units or fraction thereof*

4.3.1 The testing laboratory, may, within its discretion, instruct the person who draws the sample to submit:

a) separate samples from each unit selected, or
b) one or more composite samples consisting of aliquot portions from each unit selected.

4.3.1.1 The latter course should only be applied where the product is likely to be of fairly uniform composition, for example, where the consignment to be sampled is produced from a quantity of properly bulked milk, and where variations in composition from unit to unit are, therefore, small. Where there is a possibility of wide variations between different units, for example, a consignment of milk from individual producer, every selected unit shall be separately sampled.

4.4 Sampling from Storage Tanks and Rail and Road Milk Tankers

The method of sampling of milk from storage tanks and rail and road tankers is largely governed by storage/ transport conditions.

It is, therefore, difficult to lay down any rigid procedure for the sampling, but the following is recommended:

a) In all cases, the milk in the tank/tanker shall be thoroughly mixed by a sufficiently large plunger, a mechanical agitator or by compressed air; the uniformity of the samples being determined, when necessary, by mixing till such time as complete agreement is obtained between samples taken at the manhole and at the outlet cock in respect of fat and total milk solids.

   NOTE — When a plunger (*see* **4.6.1**) is used for mixing the milk in rail or road milk tankers, a convenient and satisfactory method is to insert the plunger in the manhole, the operator sitting astride or standing on top of the tanker. The plunger is thrust forward and pulled back, thrust downwards and pulled back and thrust backwards and pulled back. The cycle of operations should be repeated for at least 15 min.

b) After proper mixing of the milk, the sample may be taken from the tank, removed through the stopcock in the tank door, or from a valve in the discharge line from the tank as it is being emptied.

4.5 Composite Milk Samples for Fat Test

Suppliers of milk are often paid for milk on the basis of fat test (*see* **13**). The determination of fat contents of the suppliers' daily deliveries is laborious and expensive. Composite samples of the suppliers' milk are taken over a period and then tested. The volume of the individual composite sample shall be not less than 175 ml and it shall be collected during the agreed period by placing into the patron's composite sample bottle proportionate amounts of the suppliers' daily delivery. For preserving the composite sample, 0.1 ml of 36 percent formaldehyde for 25 ml of milk may be used. The bottle containing the composite milk sample shall be tightly stoppered to prevent evaporation and kept in a locker, away from light, till required for analysis. The sample shall be analysed on the same day as the last portion of milk is transferred to the composite sample bottle.

NOTE — Each time when fresh sample of milk is added, the sample shall be mixed by rotating the bottle to prevent the formation of solid cream layer or cream plug.

4.6 Appliances for Sampling

The following appliances are required for sampling:

a) Plungers (*see* Fig. 1 and Fig. 2);
b) Sampling dippers (*see* Fig. 3); and
c) Sampling tube.

They shall preferably be made of stainless steel, but adequately tinned iron may also be used. If solder is employed, it shall be capable of withstanding a sterilizing temperature of 180°C. All surfaces shall be smooth and free from crevices or projections.

4.6.1 *Plungers*

Plungers shall have sufficient area to produce adequate disturbance of the product, and sufficiently light in weight for the operator to be able to move them rapidly through the liquid. In view of the differing shapes and sizes of containers, no specific design of plunger can be recommended for all purposes. A form of plunger recommended as being suitable for the mixing of milk in buckets or in cans (*see* Fig. 1) consists of a disc 150 mm in diameter, perforated with six holes each 12.5 mm in diameter on a pitch circle of 100 mm diameter, the disc being fixed centrally to a metal rod, the other end of which forms a loop handle. The length of the rod, including the handle, should be approximately 1 m. A suitable plunger (*see* Fig. 2) for use with road and rail tanks has a rod not less than 1.8 m in length and is fitted with a disc 300 mm in diameter perforated with twelve holes each 30 mm in diameter on a pitch circle of 225 mm diameter.

4.6.2 *Sampling Dippers*

Sampling dippers (*see* Fig. 3) shall be fitted with a solid handle at least 150 mm long. The capacity of the sampling dipper shall be not less than 80 ml. It is advantageous to have a dip and have handle bent over. The tapered form of the cup permits nesting of the sampling dippers. The arc of the inside bottom corner of the sampling dipper shall be defined to assure proper cleaning. The body of the sampling dipper shall be of one-piece construction with no seams, overlaps, rivets, or sharp corners.

NOTE — When approximate quantity of milk is to be measured, dipping measures of convenient quantities may also be used.

4.6.3 *Sampling Tube*

Straight seamless metal tube about 600 mm long, of 6 mm inside diameter and about 1.6 mm thickness may be used for sampling, where convenient.

4.7 Sample Bottles

The sample bottles shall be made of good quality glass, suitable for sterilization. The sample bottle shall be wide mouthed, round with sloping sides on the pattern of the milk bottles. Bottles used for collecting samples for chemical analysis shall be provided with well-fitting caps, or bark corks. Bottles for collecting samples for bacteriological examination shall be glass stoppered.

4.7.1 The capacity of the sample bottle shall be 100, 150 or 250 ml. The size of the sample bottle selected for taking a sample shall be such that after containing the quantity of milk required for analysis, only a small space would be left for efficient mixing of the sample as a larger space would allow the fat to churn during transit.

4.7.1.1 When samples are collected for bacteriological examination, it is desirable to avoid air-space by filling the bottles to the top, leaving however, sufficient space to allow for expansion of the rubber stopper.

4.7.2 Bark corks shall not be used for closing milk sample bottles for bacteriological examination.

4.7.3 Sample bottles containing milk which are to be examined for flavour subsequently shall be closed with

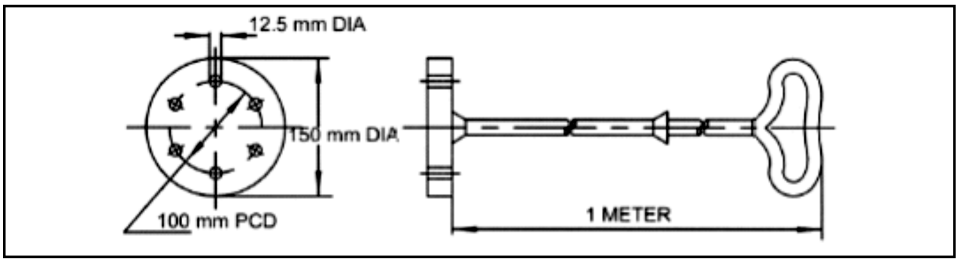


FIG. 1 PLUNGER FOR CANS AND BUCKETS

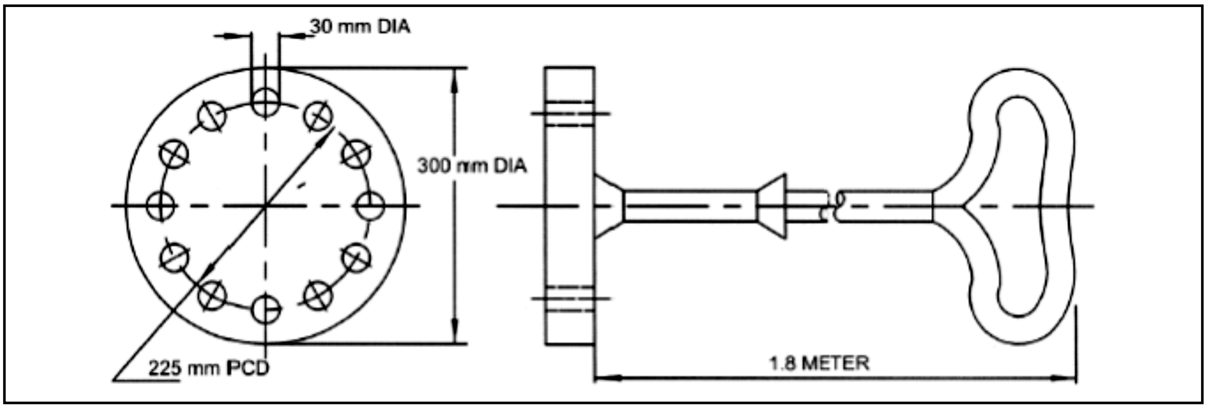


FIG. 2 PLUNGER FOR ROAD AND RAIL TANKS

grease-proof, non-absorbent stopper so that no deleterious odour or taste is imparted.

NOTE — Alternatively, for collecting samples for chemical analysis suitable plastic bottles of above capacities may also be used.

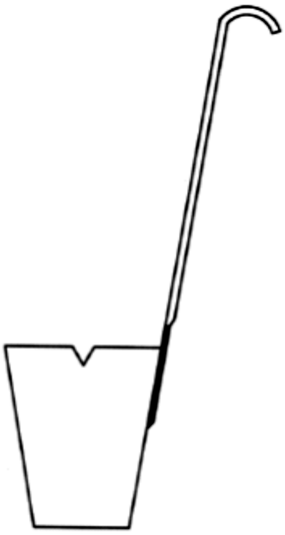


FIG. 3 SAMPLING DIPPER

4.8 Labelling of Samples

Each sample container shall be sealed air-tight after filling and marked with particulars regarding the purpose of sampling, the name of the supplier or other particulars of the stock, the date and time of sampling, the nature of preservatives, if any, added and any other relevant information. Samples for bacteriological examination shall be marked distinctively.

4.9 Transport of Samples

Milk samples which are to be examined for flavour (*see* **5**) shall be protected from light and shall not be exposed to odours which may be absorbed during transport.

a) *Samples for Chemical Examination* — It is desirable that samples of milk for chemical examination are delivered for testing on the same day they are taken. The samples shall be stored in a refrigerator at a temperature of 0 to 5°C. Where this is not possible, adequate precautions shall be taken to prevent deterioration and exposure to high temperature and light during transit. In some cases, formaldehyde may be added as a preservative to prevent deterioration, provided it does not interfere with the subsequent analysis. When formaldehyde has been added, this fact and the quantity added shall be indicated on the lable.

   NOTE — If any, sample is to be used for cryoscopic examination, mercuric chloride shall be permissible as a preservative, provided the bottle is properly labelled as 'POISON'.

b) If the tests are to determine the bacteriological quality, the milk samples shall be chilled immediately and maintained at a temperature not exceeding 4.5°C. If the interval between sampling and examination exceeds 4 h, the time of sampling and examination should be recorded on the analytical report. Generally the samples should be examined within 4 h of collection. The results of analysis of any sample, the temperature of which has exceeded 7.0°C during a storage period of 4 h, may be unreliable. At a storage temperature of 0.0 to 4.5°C no detectable increase in bacterial counts will occur within 24 h.

5 ORGANOLEPTIC TEST AND TEMPERATURE

5.0 Judging the quality of milk by its taste and smell requires considerable skill which could only be acquired by practice. Organoleptic tests are used in all dairies and an experienced person can pick out bad samples with a high degree of accuracy.

5.1 Adopt the following procedure on the receiving platform:

a) Smell the milk in the container immediately after removing the lid. In case of foul or abnormal smell, reject the milk or hold over for subjection to confirmatory test;

b) Observe the colour of the milk. If abnormal in colour, it should be regarded with suspicion; and

c) Examine the milk for the following taints:

   1) Those due to developed acidity. This is the most important factor to be examined when grading milk by organoleptic test.

   2) Those due to feed, or exposure of milk to the atmosphere of the stable.

   3) Extraneous matter which might gain access to milk after milking.

   4) Oxidized flavour due to exposure of milk to light or metallic contamination from untinned containers.

d) Determine the temperature of milk with a standard thermometer. Bulk raw milk, when received from a chilling station in the factory shall not have a temperature more than 7°C.

6 SEDIMENT TEST

6.0 Sediment test on raw milk reveals the extent to which visible insoluble matter has gained entrance to the milk and the extent to which such material has not been removed from milk by single service strainers. Despite the limitations as to the interpretation that may be applied when visible sediment is or is not detected in milk, the sediment test presents a simple, rapid and a quantitative measure of indicating the cleanliness of milk with respect to visible dirt. The test is carried out by allowing a measured quantity of milk to pass through a fixed area of a filter disc and comparing the sediment left with the prepared standards.

6.1 Apparatus

a) *Sediment Tester* — with filtering surface 2.5 cm in diameter.

b) *White Lintine Cotton Discs* — 32 mm in diameter, exposed filtration area 28 mm in diameter.

c) *Sampling Dipper* — of 500 ml capacity for sampling from milk cans or weigh vats.

d) *Sieves* — two, one coarse corresponding to 850 micron IS Sieve and the other fine corresponding to 425 micron IS Sieve [*see* IS 460 (Part 1)].

e) *Sediment Disc Ratings* — showing 0.0, 0.2, 0.5, 1.0, 2.0, 3.0 mg sediment, or higher concentration as required, per 500 ml of milk.

6.1.1 Since sediment encountered in different localities may vary in composition, density, colour and other physical characteristics thus giving rise to variations in the appearance of the discs, it is recommended that the standard sediment discs be prepared as follows:

Make a uniform mixture of oven-dried (100°C) materials which meet the following screening specifications :

Cow or buffalo dung passing through fine sieve [*see* **6.1** (d)]	53 parts
Cow or buffalo dung passing through the coarse sieve but retained on the fine one	10 parts
Garden soil passing through the fine sieve	27 parts
Charcoal passing through the fine sieve	8 parts
Charcoal passing through the coarse sieve but retained on the fine one	2 parts
Total	100 parts

Accurately weigh 0.1 g of the above mixture and transfer to a 1 000 ml flask, using 50 percent sugar solution to wash all fine particles down into the flask. Make the volume up to the mark with more of the sugar solution after most of fine particles have been wetted by shaking the half-filled flask thoroughly several times. After the volume is made up to the mark, shake the contents of the flask vigorously every 5 min for sufficient time (for 30 min to 1 h) to saturate particles thoroughly. When particles have been thoroughly wetted, it will be noted that the sugar solution will hold them evenly in suspension and the mixture is ready for use in making the standard discs.

On the basis of 0.1 g per 1 000 ml, 10 ml of the sugar solution contains 1 mg of the sediment. Make test discs with one of the usual sediment testers, using varying volumes of the sediment suspension. Place 150 ml or more of filtered skimmed milk in the sediment tester and add varying volumes of the sediment suspension. After forcing the milk through the disc, run through a small quantity of filtered skimmed milk to obtain a more even distribution of the sediment on the disc.

Remove the discs from the tester, mount them permanently on a stiff paper, allow to dry, and then make permanent by spraying with a strong disinfectant such as corrosive sublimate. Below each mounted standard disc on the paper, note the quantity of dried material that the dirt or filth on the disc represents.

6.2 Procedure

Take a milk sample from well-stirred cans or vats of milk with the help of the sampling dipper [*see* **6.1** (c)]. Measure the quantity of milk used with reasonable accuracy. Filter the milk through a properly adjusted, firm lintine cotton disc (rough side facing milk) held in the sediment tester so that a filtration area of 28 mm in diameter is exposed. Compare the sediment disc with the prepared sediment standard discs and record the sediment score.

6.3 Interpretation

For the purpose of comparison, it is convenient to use about five prepared standard discs so as to classify the milk with respect to its sediment content in accordance with the specific requirement of the dairy, or the milk collection depot. For the former, five discs showing 0.0, 0.2, 0.5, 1.0 and 2.0 mg may suffice. Under rural conditions, discs showing 0.0, 0.5, 2.0, 5.0 and 7.0 mg sediment may be more convenient to start with. In either case, no attempt shall be made to estimate the degree of sediment in milk in more than five classes, for example, Excellent, Good, Fair, Bad and Very Bad.

No attempt shall be made to grade as sediment any hair, flies, pieces of hay or straw, or any large particles of dirt. These shall be reported separately.

The presence of appreciable sediment in unprocessed milk supplies indicates careless or insanitary dairy farm practice. However, the lack of sediment is not always indicative of ideal conditions, since visible sediment may be readily removed by straining at the dairy farm. Milk that has been divested of visible sediment by straining retains essentially the bacterial contamination incidental to the original sediment.

7 DETERMINATION OF *p*H

7.0 General

The *p*H value, or hydrogen ion concentration, gives a measure of the true acidity of milk. The relationship between *p*H and acidity of milk is only approximate. In normal cow and buffalo milk, the *p*H ranges from 6.6 to 6.8. The value is reduced by the development of acidity. On the other hand, the *p*H value of milk from animals suffering from mastitis is alkaline in reaction, the value being over 7.0. The *p*H test is mainly used for the detection of abnormal mastitis milk. The *p*H of milk may be determined rapidly by using the indicator strips.

7.1 Indicator Strips

Indicator paper strips or discs are made by soaking strips of absorbent paper in a suitable indicator and drying them. A rough estimate of *p*H is obtained by dipping a strip of the prepared paper in milk and observing the colour. Bromocresol purple (*p*H range 5.2 to 6.8 — colour changes from yellow to purple) and bromothymol blue (*p*H range 6.0 to 7.6 — colour changes from straw-yellow to bluish-green), are commonly used as indicators. Both narrow and wide-range ready-made indicator papers are available over the *p*H range 2.0 to 10.5.

NOTE — Indicator paper strips shall always be kept in a closed glass bottle and in a dry condition.

7.2 Interpretation

In normal milk the *p*H is well below 6.9. On an average cow milk gives a *p*H of 6.6, and buffalo milk 6.8. Milk of *p*H over 6.9 should be regarded with suspicion as indication of some diseases of the udder, or of late lactation milk.

8 CLOT-ON-BOILING (C.O.B.) TEST

8.0 This is a quick test to determine developed acidity and the suitability of milk for processing.

8.1 Apparatus

a) *Test-Tubes* — 15.0 cm × 1.9 cm, preferably with a mark at 5 ml.

b) *Water-Bath*

8.2 Procedure

Transfer 5 ml of the sample to the test-tube and smell. Place the tube in a boiling water-bath and hold for about 5 min, and smell again for any acidic flavour. Remove the tube and rotate it in an almost horizontal position and examine the film of milk or side of the test-tube for any precipitated particles. The formation of lot is indicative of a positive test.

8.3 Interpretation

The principal features of the boiling test are speed and definiteness of results. Milk either remains unchanged or coagulates. Milk which gives a positive C.O.B. test has an acidity generally above 0.17 percent (as lactic acid) and is not suitable for distribution as liquid milk or for processing.

9 ALCOHOL TEST

9.0 General

The alcohol test is used for rapid assessment of stability of milk to processing, particularly for condensing, and sterilization. The alcohol test is useful as an indication of the mineral balance of milk and not so much as an index of developed acidity. The test aids in detecting abnormal milk such as colostrum milk from animals in late lactation, milk from animals suffering from mastitis, and milk in which the mineral balance has been disturbed.

9.1 Apparatus

a) *Test-Tubes* — 15 cm × 1.9 cm, preferably with graduation marks at 5 and 10 ml.
b) *Measure for Alcohol* — for 5 ml.

9.2 Reagent

Ethyl alcohol, 68 percent by weight or 75 percent by volume (density 0.867 5 g/ml at 27°C).

9.3 Procedure

Place 5 ml of milk in a test tube and add an equal quantity of alcohol. Mix the contents of the test-tube by inverting several times. Note any flakes or clot. The presence of a flake or clot denotes a positive test.

9.4 Interpretation

A negative test indicates low acidity and good heat stability of the milk sample. Milk showing positive test is not considered suitable for the manufacture of evaporated milk which has to be sterilized to ensure its keeping quality.

10 HALF-HOUR METHYLENE BLUE REDUCTION (M.B.R.) TEST

10.0 General

The length of time taken by milk to decolourize methylene blue is a fairly good measure of its bacterial content, and hence of its sanitary and keeping quality.

10.1 Apparatus

a) *Sterile Test-Tubes Without Rims* — 150 mm × 16 mm, internal diameter 13.5 ± 0.5 mm accurately marked at 10 ml. If not used directly after sterilization, they shall be kept in closed boxes protected from dust.
b) *Sterilized Rubber Stoppers* — for closing the test-tubes. The stoppers are sterilized by immersing in a boiling water-bath for not less than 10 min.
c) *Sterile 1-ml Pipettes* — straight-sided, blow-out delivery pipettes for measuring the dye solution may preferably comply with the following specification :

Overall length	300 mm
External diameter	7.5 to 8.5 ml
Internal diameter	2.6 to 3.0 mm
Graduation	One mark only at 1 ml level
Distance of graduation from tip	140 to 180 mm
Distance of graduation from top	120 to 160 mm

The pipettes shall also be calibrated to deliver 1 ml of water at 27°C when the contents are blown out with the tip touching the side of the vessel, three seconds allowed for drainage and the accumulated drop then blown out. No pipette should have an error of more than ± 2 percent, that is, the amount delivered should be between 0.98 mlh and 1.02 ml.

d) *Sterile 10-ml Pipettes* — straight-sided, blow-out type.
e) *Sampling Dippers* — These shall be sterilized by keeping in boiling water for 30 min. Water shall be changed at frequent intervals when a series of samples are to be examined.
f) *Pipette Case* — of metal.
g) *Water-Bath* — maintained at 37.5 ± 0.5°C, fitted with a cover to exclude light, and containing a metal rack, designed to hold test-tubes when immersed in water. The water-bath shall preferably be thermostatically controlled. The level of water in the bath shall be maintained above the level of the milk in the tubes. The interior of the bath shall be completely dark.
h) *Clock or Watch*
j) *Hot Air Oven Autoclave or Steam-Sterilizer*
k) *Wire Baskets* — for holding test-tubes.
m) *Glass Marking Pencil*
n) *Bunsen Burner, or Spirit Lamp*
p) *Glass Still* — for preparing distilled water,
q) *Comparator with Standard Resazurin Disc* — The comparator may be provided with artificial daylight source of illumination.
r) *Sterile 50-ml Measuring Flask or Cylinder*

10.2 Reagent

10.2.1 *Methylene Blue Solution* — Prepare a standard solution of methylene blue by dissolving one of the good quality methylene blue thiocyanate tablets in 200 ml of cold, sterile, glass-distilled water in a sterile flask. It is preferable to allow the mixture to stand several hours to ensure complete solution. Depending on the nature of the methylene blue tablets used, sometimes the stock solution is further diluted with 800 ml of sterile glass-distilled water. A concentration of 1 part of methylene blue thiocyanate in 300 000 parts of milk is used to obtain satisfactory results. The solution shall be stored in a sterile glass-stoppered amber-coloured bottle in a dark place, and at no time exposed to light. The solution remains stable in the dark for a considerable time but no stock solution more than two months old shall be used.

The amount of methylene blue required for a day's work shall be poured off from the stock bottle into a suitable glass container. On no account shall the pipette used for transferring the methylene blue solution to the tubes of milk be introduced into the stock bottle. Moreover, if at any time during the filling of the tubes the methylene blue solution should become contaminated with milk carried into it by a pipette which has inadvertently come into contact with the milk, the methylene blue solution shall be immediately discarded and replaced by a fresh stock.

10.3 Procedure

Thoroughly mix the sample of the milk by inverting and shaking the sample bottle and then pour the milk in the test-tube up to the 10 ml mark. While doing this, remove the stopper or cap of the bottle under aseptic conditions, the pouring-lip of the bottle and the mouth of the test-tube being flamed and then pour the milk rapidly into the tube up to the 10 ml mark. While pouring into the tube, take care to leave one side of the interior unwetted with milk. Add 1 ml of methylene blue solution to the tube from a pipette taking care that the pipette does not come into contact with any of the milk in the tube or with the wetted side of the interior of the tube. If this occurs, discard the pipette immediately. During delivery, hold the tip of the pipette against the dry side of the tube about 1 to 2 cm above the level of the milk and expel the methylene blue solution by blowing with the mouth or by means of a jet in the pipette. After the lapse of 3 s, blow out the solution remaining in the tip of the pipette and withdraw the pipette. Close the tube with a Sterile rubber stopper held and inserted by sterile forceps or by the tips of the fingers on the extreme upper end. On no account allow the fingers to come into contact with the mouth of the test-tube or with the end of the stopper which comes into contact with the test-tube. Invert the tube slowly once or twice so that the whole column of contained air rises above the level of the milk and then within 5 min, place the tube in the water-bath.

Put up the following control tubes with each batch:

a) 10 ml of mixed milk + 1 ml of tap water, and
b) 10 ml of mixed milk + 1 ml of methylene blue solution.

The milk for the control tubes shall consist of a mixture of milks, preferably from several producers so as to have an average fat content and colour. Fit the control tubes (a) and (b) with stoppers and immerse for 3 min in boiling water in order to destroy the natural reducing system present in the milk. Comparison of the experimental tubes with control tube (b) will show when decolourization begins and comparison with control tube (a) will show when it is complete.

Inspect the tube after 30 min. Regard the milk as decolourized when the whole column of milk is completely decolourized or is completely decolourized up to within 5 mm of the surface. If a trace of colour persists at the bottom of the tube and does not extend upwards for more than 5 mm, it may be ignored. Record the time at which decolourization is observed. Where a tube is found not to be decolourized within 30 min, the sample satisfies the test.

10.4 Precautions

The following precautions shall be taken:

a) It is important that the methylene blue solution when not in use should be kept in the dark: it shall at no time be exposed to sunlight.
b) It is essential that the interior of the water-bath during the progress of the tests shall be completely dark since sunlight, diffused daylight and even artificial light catalyse the reduction of methylene blue.
c) The sterilization of the rubber stoppers for the test-tube and their subsequent satisfactory manipulation can be facilitated by employing a simple rack for holding a large number of rubber stoppers immersed in a suitable vessel of boiling water.
d) The precautions against the contamination of the milk sample described in the method for carrying out the test shall be carefully observed.

10.5 Interpretation

The samples which show complete decolourization of blue colour on incubation for 30 min or less shall not be suitable for acceptance.

11 DIRECT MICROSCOPIC COUNT

11.0 General

The direct microscopic method consists of examining, under a compound microscope, stained films of a measured volume of milk spread on glass slides over specified area. It enables the rapid estimation of the total bacterial population of a sample of milk and also reveals useful information for tracing the sources of contamination in milk. The microscope is first calibrated so that the exact area of the microscopic field is known. Then a measured quantity of the milk (0.01 ml) spread over a measured area (1 cm^2) on a clean glass slide so that each microscopic field examined represents a quantitative aliquot of the sample. The milk is allowed to dry, and the film is defatted, fixed and stained with a suitable dye. The average number of bacteria per microscopic field is determined after examining between 5 and 60 fields, depending on the microscopic factor and the number of bacteria per field. Either the total cells or clumps can be counted, but the clump count agrees more closely with results obtained by the agar plate method than does the total cell count.

This method offers the most rapid technique for determining the extent of bacterial contamination in milk and requires 10 to 15 min for examining one sample. It has been used for the classification of raw milk samples into different grades on the basis of clump counts, for screening milk samples for 'acceptable' quality, and for obtaining information regarding likely sources of contamination. The microscopic appearance (types and arrangement of cells) of the milk film will give indication of any udder infections as well as the cause of high counts due to utensil contamination or inadequate cooling.

Estimates of bacterial counts by this method may be expected to be relatively more accurate in the case of poor quality milks having high bacterial counts. In the case of low count samples, examination of a large number of fields is necessary to obtain some accuracy in the estimates. Other sources of error in this method are those caused by (a) variable distribution of bacteria in films, especially those prepared from samples of high sanitary quality; (b) inaccurate measurement of 0.01 ml quantities and faulty preparation and staining of slides; (c) failure of this method to distinguish between the dead and living organisms; and (d) mistakes in observation and calculation.

11.1 Apparatus

11.1.1 *Microscope Slides* (optional sizes 2.5 cm × 7.5 cm, 5.0 cm × 7.5 cm or 5.0 cm × 11.25 cm) of clear glass. They may be either — (a) plain or with etched margins to permit unmistakable identification of films; or (b) with clear, circular one square centimetre areas (diameter approximately 11.28 mm) delineated on each, with remaining area etched. The slides should be clean and dry before use and should not contain any finger-prints or other residues on areas where milk films are to be placed.

New slides may be cleaned by soaking in strong cleaning solution (bichromate-sulphuric acid solution prepared by dissolving 50 g sodium bichromate in 200 ml of water in glass or earthen container and then adding cautiously 300 ml of concentrated sulphuric-acid of commercial grade), rinsing in flowing tap water and then in distilled water. Used slides should be soaked in hot or boiling alkaline detergent solution until all residues are removed, then rinsed in flowing tap water, dried and properly stored for reuse. Slides may be stored submerged in chloroform or alcohol and drained and dried at the time of use.

11.1.2 *Guide Plates,* special glass plates or cardboard guides, convenient size 5.0 cm × 11.25 cm with 16 or 24 round or square areas, each one square centimetre, inscribed thereon, for use beneath plain slides.

11.1.3 *Pipette or Syringe for Transfer of 0.01 ml of Milk*

a) Pipettes (Breed) shall be calibrated to deliver 0.01 ml of milk. They shall be of straight thick wall, capillary tubing with bore of such diameter that the single graduation mark is 40 to 60 mm from tip, and the tip blunt, and formed so as to discharge milk cleanly. Pipette calibrated to contain 0.139 5 g (approximately 140 mg) of mercury will discharge 0.01 ml of milk at 20°C.

   When not in use, the pipettes may be kept submerged in and the bores filled with suitable soapless detergent or strong cleaning solution. Before use, the bore and exterior are thoroughly rinsed in clean water until free from detergent or cleaning solution. While using the same pipettes for a number of samples, the graduated portion of the instrument is rinsed each time in clean water (at 25 to 35°C) by dipping the tip slightly beneath surface and repeatedly drawing in and expelling water.

b) Alternatively, a metal syringe, capable of transferring rapidly 0.01 ml of milk with semi-automatic, spring-actuated plunger, with stainless steel piston sliding in close fitting, stainless steel measuring tube, with an adjustable set-screw to permit presetting instrument for repeated accurate measurements of 0.01 ml, may be used. Accuracy of adjustment should be determined by analysis before use to assure 0.01 ml deliveries (average 0.0103 g). It should be cleaned only in soapless detergents and fat solvents.

11.1.4 *Needle, Bent-Point,* suitable for spreading milk over an area of one square centimetre.

11.1.5 *Drying Device,* level surface at 40 to 45°C, clean, dust-free, insect proof (cabinet or metal box over electric bulb is satisfactory).

11.1.6 *Forceps or Slide Holders,* suitable for dipping and holding slides.

11.1.7 *Trays or Jars,* equipped with tight-fitting covers, of size convenient for holding solvents and stains and submerging slides held in partitioned racks.

11.1.8 *Slide Storage Boxes, Cases or Files*

11.1.9 *Compound Microscope,* binocular (preferably) or monocular with 1.8 mm oil-immersion objective, sub-stage and condenser, mechanical stage, and oculars for different magnifications. A substage lamp or separate microscope lamp may be used as a source of artificial illumination in preference to day light, for microscopic examination.

11.1.10 *Stage Micrometer,* slide ruled in 0.1 and 0.01 mm.

11.2 Reagents

11.2.1 *Xylene,* or any other fat solvent may also be used.

11.2.2 *Ethyl Alcohol,* 95 percent.

11.2.3 *Immersion Oil,* of refractive index 1.51 to 1.52 at 20°C.

11.2.4 *Alcoholic Methylene Blue Chloride Solution,* prepared by adding 0.6 g methylene blue chloride to 100 ml of 95 percent ethyl alcohol. The mixture is shaken for some time, left at room temperature for 24 to 48 hours shaking at intervals until the dye is completely dissolved, and then stored in clean, tightly closed container. Alternatively, Newman's stain prepared by dissolving methylene blue powder in a mixture of glacial acetic acid, tetrachlorethane and ethyl alcohol in the following proportions may be used:

Methylene blue chloride	1 g
Glacial acetic acid	6 ml
Tetrachlorethane	40 ml
Ethyl alcohol (95 percent)	54 ml

11.3 Procedure

11.3.1 *Determination of Microscopic Factor* — This factor is to be determined for each microscope in the following manner:

Focus on the scale of the stage micrometer with the 16 mm objective. Place a drop of immersion oil on the micrometer scale and focus with the 1.8 mm objective. Move the stage micrometer until one end of the scale arc is at the edge of the field. Count the number of small spaces (0.01 mm each) in the diameter of the field and determine the diameter of the field, which may range from 0.146 to 0.206 mm depending on the length of the draw tube of the microscope (usually American microscopes are adjusted to 160 mm and Continental microscopes to 170 mm) and the objective and ocular used. The microscopic factor (*MF*) is given by the formula:

$$MF = \frac{10\ 000}{3.141\,6 \times r^2}$$

where r is the radius of the microscopic field.

In effect, the reciprocal of the *MF* represents the fraction of 1 ml of milk actually seen in one microscopic field. Thus, if N is the average number of cells counted in one microscopic field, the total counts per ml of milk will be *NX* microscopic factor, using a 10 *X* ocular with 1.8 mm oil-immersion objective, the microscope can be adjusted to give a microscopic factor in round numbers ranging from 3 000 000 to 5 000 000 depending upon the tube length of the microscope. Once this microscopic factor has been calculated for a definite tube length and combination of objective and ocular, it can be used as a constant factor for determining direct microscopic counts of all milk samples, provided the same adjustment of the microscope is followed throughout.

11.3.2 *Preparation of Milk Film on Microscopic Slides* — Mix the sample of milk thoroughly by shaking. If single 0.01-ml pipette is used, rinse it thoroughly in sterile water (25 to 35°C) between samples. Dip the tip of the pipette into the sample of milk and draw in and expel several times to remove traces of rinse water. Draw milk into the pipette above the graduation mark, wipe the exterior of the pipette with clean, dry paper or cloth towel, and adjust the volume of the sample to exactly 0.01-ml mark. Touch the tip of the pipette to the centre of a 1 cm^2 area on a slide and expel the entire volume of milk. With a flamed bent-point needle, promptly spread the portion of milk uniformly over the entire 1 cm^2 area on the slide. Wipe needle between samples on a clean dry tissue or towel.

If a metal syringe is used, rinse the tube as described above and with draw milk by holding the tip beneath the surface and releasing the plunge fully. Remove excess milk from the exterior of the tip with clean paper tissue or cloth. Holding the instrument nearly vertical, place the tip near the centre of 1 cm^2 area on the slide and expel the 0.01 ml portion of milk. With the plunger, held fully depressed and with syringe nearly vertical, spread the milk with the tip of extended piston rod over the entire 1 cm^2. Legibly and indelibly identify each sample on slide, using number or other symbol on margin of slide. After spreading, dry films at 40 to 45°C within 5 min on level surface protected from dust and insects. Rapid heating may cause the film to crack and peel out during later treatments.

11.3.3 *Defatting, Fixing and Staining the Films* — After the films are dried, submerge the slides in clear xylene (or other suitable fat solvent) for 1 or 2 min. Drain and air-dry the films. Fix the film in 90 to 95 percent ethyl alcohol for 1 min either by submerging the slide in alcohol kept in a jar or by gently pouring the alcohol over the film. Allow the slide to drain and air-dry completely. Dip the slides edgewise in alcoholic methylene blue staining solution (in a jar) for 1 or 2 min. Slowly remove slides, allowing a few seconds for stain to drain into staining jar. Wash in fresh tap-water in a beaker or large staining jar by raising and lowering edgewise a number of times to remove excess stain without impairing milk films. The cells will be stained deep blue against a faint blue background. Allow stained slides to drain and air-dry gradually.

Alternatively, the slide containing the dried milk film, can be directly dipped in Newman's stain for to 1 min without the preliminary treatments for defatting and fixing. The composition of Newman's stain is intended to remove fat, fix the cells and stain the organisms in one single operation. If the film is over-stained, rinse in water and decolourize with alcohol slightly. The background will be faintly blue while the cells will be stained deep blue.

NOTE — There are a number of other staining procedures but the two methods described above have been found to be quite satisfactory.

11.3.4 *Microscopic Examination of Stained Films* — Place one drop of immersion oil on film and examine under the oil-immersion objective. Count the single organisms or isolated clumps on a number of microscopic fields. Any isolated single cell, pair of cells or clump of cells is treated as a 'clump'. The fields counted shall be selected at random and represent all parts of the film. The number of fields to be counted is determined by the density of bacterial clumps in the field in the following manner:

> If the average number of clumps per field is under 0.5, 0.5 to 1, 1 to 10 and 10 to 30, the number of fields to be counted will be 50, 25, 10 and 5 respectively. If the number of clumps per field is over 30, then the counter is recorded as uncountable. In such cases, if the actual count is required, the milk sample will have to be diluted suitably and then the microscopic count determined.

Calculate the average number of the clumps per field and multiply by the microscopic factor (*MF*) to give the Direct Microscopic Clump Count per ml. (Alternatively, the individual cells in each field may be counted and the results reported as total counts per ml).

11.4 Interpretation

The following are tentatively suggested for assessing the bacteriological quality of milk supplies on the basis of Direct Microscopic Clump Counts:

Direct Microscopic Clump Counts per ml	*Bacteriological Quality of Milk*
a) Less than 5 000 00	Good
b) 5 00 001 to 4 000 000	Fair
c) 4 000 001 to 20 000 000	Poor
d) Over 20 000 000	Very poor

11.4.1 A careful study of the types of organisms predominating in the milk film during microscopic examination will reveal valuable information regarding the sources of contamination in milk so that suitable advisory and regulatory measures may be undertaken to improve the quality of milk:

Microscopic Appearance	*Probable Causes of High Counts or Poor Quality*
a) Presence of many cocci and rods in clumps and patches	Improperly cleaned milk utensils
b) Excessive numbers of rod shaped bacteria (particularly spore-formers)	Dusty and dirty environment
c) Large numbers of cocci in pairs or short chains	Improper cooling of milk
d) Large numbers of leucocyte cells (generally exceeding 500 000 per ml) together with long chains of cocci	Mastitis infection

11.5 Preservation of Records

After examination of films and removal of oil (by adding a little xylene and gently removing the dissolved oil), the slides may be preserved in a special dust-free and insect-proof rack or cabinet for future reference.

12 ACIDITY

12.0 General

The titratable acidity test is employed to ascertain if milk is of such a high acidity as to reduce its keeping quality and heat stability. The acidity of milk is not a true measure of lactic acid present but in practice, gives a good indication of the quality of milk. The titrable acidity test really measures the amount of alkali which is required to change the *p*H of milk from its initial value of about 6.6 to 6.8, to the *p*H of the colour change of phenolphthalein added to milk to indicate the end point (*p*H 8.3). In practice, the titration method is employed and measures the buffering capacity of milk and not true acidity.

Due to the opacity of milk, the end point of titration is not sharp and care has to be taken to adjust the conditions to reach the same end-point. Numerous techniques are available but the one recommended in this standard is the simplest.

12.1 Apparatus

a) *White Porcelain Basins,* hemispherical, 60 ml capacity.
b) *Pipette,* 10 ml.
c) *Burette,* with soda-lime guard-tubes.

d) *Measuring Cylinder,* 25 ml.
e) *Stirring Rods,* glass, flattened at one end.

12.2 Reagents

12.2.1 *Standard Sodium Hydroxide Solution,* 0.1 N. Prepare a concentrated stock solution of sodium hydroxide by dissolving equal parts of sodium hydroxide (sticks or pellets) in equal parts of water in a flask. Tightly stopper the flask with a rubber bung and allow any insoluble sodium carbonate to settle out for 3 to 4 days. Use the clear supernatant liquid for preparing the standard 0.1 N solution. About 8 ml of stock solution is required per litre of distilled water. Standardize the prepared 0.1 N solution against standard oxalic acid solution (0.1 N).

12.2.2 *Phenolphthalein Indicator Solution* — Dissolve 1 g of phenolphthalein in 100 ml of 95 percent ethyl alcohol. Add 0.1 N sodium hydroxide solution until one drop gives a faint pink colouration. Dilute with distilled water to 200 ml.

12.2.3 *Rosaniline Acetate Solution* (*Stock Solution*) — Dissolve 0.12 g of rosaniline acetate in approximately 50 ml of rectified spirit (*see* IS 323) containing 0.5 ml of glacial acetic acid. Make up to 100 ml with rectified spirit.

12.2.4 *Rosaniline Acetate Solution* (*Bench Solution*) — Dilute 1 ml of the stock solution to 500 ml with a mixture of rectified spirit (*see* IS 323) and distilled water in equal proportions by volume.

NOTE — The stock solution and the bench solution should be stored in dark in brown bottles securely stoppered with rubber bungs.

12.3 Procedure

Thoroughly mix the milk by pouring several times from one container to another avoiding incorporation of air bubbles. Measure accurately 10 ml of milk in two porcelain basins. Add an equal volume of freshly boiled and cooled water. Add 1.0 ml of phenolphthalein indicator solution to one of the basins and to the other basin, add 1.0 ml of bench solution of rosaniline acetate. Titrate the contents of the basin to which phenolphthalein has been added, against standard sodium hydroxide solution added drop by drop from the burette until by comparison the colour matches the pink tint of the solution in the basin containing the rosaniline acetate solution. Stir vigorously throughout. The time taken for complete titration shall not exceed 20 s. The titration shall be made in north light or under illumination from a daylight lamp.

12.4 Calculation

Titratable acidity (as lactic acid per 100 ml of milk)

$$= \frac{9V_1N}{V_2}$$

where

V_1 = volume in ml of the standard sodium hydroxide required for titration;
N = normality of the standard sodium hydroxide solution; and
V_2 = volume in ml of milk taken for the test.

12.5 Interpretation

The normal range of acidity of milk varies from 0.10 to 0.17 percent lactic acid. Any value in excess of 0.17 percent can safely be reckoned as developed lactic acid.

13 DETERMINATION OF FAT

Determine fat in the sample (*see* **4.5**) according to the method prescribed in IS 1224 (Part 1).

14 DETERMINATION OF SOLIDS-NOT-FAT

Calculate the solids-not-fat according to the method prescribed in IS 10083.

15 DETECTION OF ADULTERANTS

15.1 The modes of adulteration commonly encountered in market samples are:

a) removal of fat by skimming,
b) addition of separated milk or skim milk to whole milk,
c) addition of water,
d) addition of starch and cane sugar for raising density to prevent detection of added water by lactometers,
e) addition of urea, and
f) addition of vegetable oils/foreign fat.

15.2 Detection of Skimming

An indication of the removal of excess fat from milk is given by the following:

a) Lower percentage of fat,
b) Higher density reading of the sample at 27°C, and
c) Higher ratio of solids-not-fat to fat.

15.3 Detection of Milk Mixed with Separated Milk or Skim Milk

When fresh separated milk or skim milk has been added to whole milk, it could be inferred from the following facts:

a) Lower percentage of fat (*see* **13**),
b) Higher density of the toned milk sample at 27°C,

c) Higher percentage of solids-not-fat (*see* **14**), and
d) Higher ratio of solids-not-fat to fat.

15.4 Detection of Extraneous Water

Presence of extraneous water in milk is detected by the following facts:

a) Lower percentage of fat (*see* **13**),
b) Lower density of milk at 27°C,
c) Lower percentage of solids-not-fat (*see* **16**), and
d) Depression of freezing point [*see* IS 1479 (Part 4)].

15.5 Detection of Mineral Oil

15.5.0 *General*

The presence of mineral oil in milk fat is indicated by the development of turbidity when hot distilled water is added to a freshly made alcoholic solution of the soap formed by the isolated milk fat/oil. For the detection of mineral oils in milk, milk fat needs to be separated and converted into *Ghee*.

NOTE — Sample of *Ghee* cane be prepared by taking out cream/ butter from milk sample and melting the same into *Ghee*.

15.5.1 *Apparatus*

Erlenmeyer flask, air condenser/water condenser.

15.5.2 *Reagent*

Potassium hydroxide (KOH) solution (3 parts KOH and 2 parts distilled water).

15.5.3 *Procedure*

Place 1 ml oil or melted fat in Erlenmeyer flask, add 1 ml KOH solution and 25 ml alcohol. Boil under reflux air condenser, shaking occasionally until saponification is complete (approx. 5 min). Add 25 ml water and mix.

15.5.4 *Interpretation*

In presence of >0.5 percent mineral oil on fat basis, distinct turbidity appears.

15.6 Detection of Starch

Starch or cereal flours, may be added to make up the density of milk to prevent detection of added water. The presence of starch or cereal flours is detected by the following test.

15.6.1 *Reagents*, Iodine solution (1 percent) Dissolve 2.5 g potassium iodide (KI) in 100 ml distilled water, add to it 1 g pure iodine crystals, shake well until the iodine crystals dissolve.

15.6.2 *Procedure*

Place in a test-tube about 3 ml of well-mixed sample. Bring it to boil by holding the tube over a flame. Allow to cool to room temperature. Add a drop of one percent iodine solution. Presence of starch is indicated by the appearance of a blue colour which disappears when the sample is boiled and re-appears on cooling. The test can detect presence of starch at 0.02 percent level in milk.

15.7 Detection of Cane Sugar

15.7.0 *General*

Cane sugar may be added to milk to raise the density to prevent detection of extraneous water.

15.7.1 *Procedure*

To about 15 ml of milk in a test-tube, add 3 ml of concentrated hydrochloric acid and 0.1 g of resorcinol and mix. Place the tube in boiling water-bath for 5 min.

15.7.2 *Interpretation*

A white colour or white with slight yellowish tinge is considered as negative for cane sugar and development of slight red colour to brick red colour is considered as positive for added cane sugar. The sensitivity of the test is 0.15 percent cane sugar in milk.

15.8 Determination of Maltodextrins in Milk

15.8.0 *General*

The test method is used to detect added maltodextrin in milk. The reaction is due to the formation of carbohydrate-iodine complex (brownish) in low *p*H condition.

15.8.1 *Reagents*

a) Iodine solution (1 percent) — Dissolve 2.5 g potassium iodide (KI) in 100 ml distilled water, add to it 1 g pure iodine crystals, shake well until the iodine crystals dissolve.
b) Lactic acid (5 percent) — Take 5.6 ml of lactic acid (90 percent) and dilute to 100 ml.

15.8.2 *Procedure*

Take 5 ml of milk and boil, add 0.5 ml drops of 5 percent lactic acid, cool and filter. Add 3 drops of 1 percent iodine solution in filtrate and observe the colour.

15.8.3 *Interpretation*

The presence of maltodextrin is indicated if the color changes to brown. If the colour remains golden yellow, then it will indicate absence of maltodextrin (*see* Fig. 4).

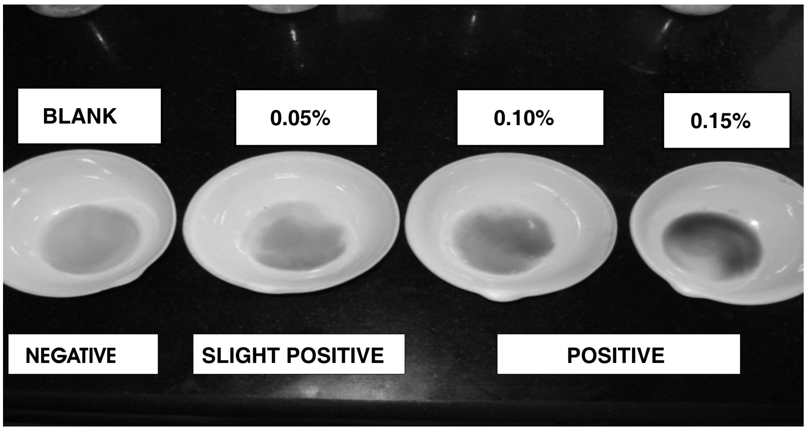


FIG. 4 CHEMICAL METHOD FOR DETERMINATION OF MALTODEXTRINS IN MILK

15.9 Detection of Glucose in Milk

15.9.1 *General*

Generally glucose is added as adulterant to increase the specific gravity of milk. General tendency of people with vested interest is to add at the rate of 0.5-1.0 percent to get sufficient increment in Lactometer reading. Therefore, this test can be successfully implemented in milk collection centers also.

15.9.2 *Apparatus* — Water-bath

15.9.3 *Reagents*

a) *Barfoed's reagent* — Dissolve 24 g cupric acetate in 450 ml boiling water and immediately add 25 ml of 8.5 percent lactic acid to the hot solution. Shake to dissolve almost all precipitate, cool and dilute with water to 500 ml. If necessary decant of filter to get a clear solution.

b) *Phosphomolybdic acid reagent* — Take 35 g ammonium molybdate and 5 g sodium tungstate in a large beaker; add 200 ml of 10 percent NaOH solution and 200 ml water. Boil vigorously (20-60 min) so as to remove nearly whole of ammonia. Check removal of ammonia with the help of red litmus paper. Cool, dilute with water to about 350 ml. Add 125 ml concentrated H3PO4 (85 percent) and dilute further to 500 ml.

15.9.4 *Procedure*

Take 1 ml of milk and add 1ml of Modified barfoed's reagent. Place it in boiling water bath for 3 minutes. Cool under tap water (2-3 min). Add 1 ml of phosphomolibdic acid. Development of deep blue colour is an indication of added glucose.

Sl No.	*Concentration, percent*	*Reaction (Reagents)*
i)	Blank (Normal milk)	Negative
ii)	0.05	Change in colour with respect to blank
iii)	0.1	Positive (deep blue colour)
iv)	0.2	Positive

NOTE — From the above results we can clearly state that sensitivity of this test starts from 0.05 percent. At 0.1 percent, we can clearly detect the presence of glucose using this test.

15.10 Detection and Determination of Urea in Milk

15.10.1 *General*

Urea content in natural milk varies from 20 mg/100 ml to 70 mg/100 ml. However, urea content above 70 mg/100 ml in milk indicates milk containing 'added urea'. The adulteration of milk by 'added urea' which serves to increase the total solids content of milk, is detected and determined by the following test.

15.10.2 *Detection of Urea in Milk*

15.10.2.1 *Reagents*

a) *p-Dimethyl amino benzaldehyde solution (DMAB), 1.6 percent (w/v)* — Dissolve 1.6 g DMAB in 100 ml ethyl alcohol and add 10 ml concentrated hydrochloric acid.

b) *Phosphate buffer, p*H 7.0 — 3.403 g of anhydrous potassium dihydrogen orthophosphate and 4.355 g of anhydrous dipotassium monohydrogen orthophosphate are dissolved in distilled water and the volume is made to 1 litre.

c) *Trichloroacetic acid* (*TCA*), *24 percent* (*m*/*v*) — 24 g of TCA is dissolved in distilled water and the total volume is made to 100 ml.

d) *Diluting reagent* — Equal volumes of 24 percent TCA and phosphate buffer (*p*H 7.0) are mixed to make the diluting reagent.

15.10.2.2 *Procedure*

Take 5 ml of milk sample and add 5 ml of 1.6 percent DMAB reagent and mix well. For comparison, prepare a control, that is, milk sample containing no added urea. A distinct yellow colour if observed in milk sample indicates milk containing 'added urea'. The control, however, would show a slight yellow colour due to the presence of natural urea in milk.

15.10.3 *Determination of Urea in Milk*

15.10.3.1 *Preparation of standard urea solution (1 mg/ ml)*

Weigh 100 mg of urea and dissolve in phosphate buffer of *p*H 7.0 and make up the volume to 100 ml with phosphate buffer.

15.10.3.2 *Procedure*

Take 10 ml of milk sample and mix with 10 ml of trichloroacetic acid (24 percent) to precipitate the proteins and then filter. Treat 5 ml of the filtrate with 5 ml of para-dimethyl amino benzaldehyde (DMAB) reagent to develop the colour. Prepare a blank by taking 5 ml of diluting reagent and treating it with 5 ml of DMAB reagent. Measure the optical density of the yellow colour at 425 nm in a spectrophotometer.

15.10.3.3 *Calculation*

Prepare standard curve by taking known concentrations of urea and treating with DMAB as above. Calculate the amount of urea in milk from the standard curve.

15.11 Detection of Vegetable Oils/Foreign Fat in Milk

15.11.1 *Isolation of Fat Using Modified Gerber Method*

Take a specially designed dual purpose milk butyrometer having both ends open. Close the stem side opening of the butyrometer with a good quality acid-resistant silicon cork. Then add, in sequence, 10 ml of Gerber sulphuric acid (90-91 percent, *v/v*), 10.75 ml of milk sample and 1 ml of iso-amyl alcohol from the neck side opening. Close the neck side of the butyrometer with a lock stopper. Mix the contents of the butyrometer properly to dissolve the proteins and centrifuge at 1 100 rpm for 5 min to get a clear fat column in the stem of the butyrometer, as done in the usual Gerber fat test. Read the fat percentage from the butyrometer column.

15.11.2 *Determination of Butyro-Refractometer (B.R.) Reading*

Remove the silicon cork and take out a little quantity of fat from the stem of the butyrometer with the help of a syringe having a thin and narrow gauge teflon tubing attached to the niddle. Apply the liquid fat between the prisms of the butyro-refractometer maintained at 40°C. Wait for 2 min and note down the observed B.R. reading.

15.11.3 *Calculation*

Calculate B.R. reading of the isolated fat as follows:

Corrected B.R. = 1.08 × Observed B.R.

15.11.4 *Interpretation*

Butyro-refractometer reading of milk fat exceeding 45.0, indicates the presence of vegetable oils/foreign fat.

15.11.5 *Precautions*

Fat column should be clear and transparent. If blackening occurs due to charring, repeat the experiment to get clear fat, as B.R. reading is affected due to blackening of the fat column.

15.11.6 *Determination of Reichert Meissel (RM) Value*

In case of doubtful Butyro-refractometer (B.R.) reading the Reichert Meissel (RM) value of milk fat isolated by heat-clarification method may be determined by the procedure prescribed in IS 3508. The RM value of isolated fat should be as per the requirement given for ghee (*see* IS 16326).

15.12 Detection of Sulphates in Milk

15.12.0 *General*

The test method is used to detect added sulphates in milk to increase the solids-not-fat (SNF) content. Soluble sulphates present in filtrate forms barium sulphate ($BaSO_4$) with barium chloride ($BaCl_2$) solution and will give precipitate of white color.

15.12.1 *Reagents*

Five percent lactic acid solution, 1 percent barium chloride solution.

15.12.2 *Procedure*

Take 20 ml of milk sample; add 1 ml of 5 percent lactic acid, boil and cool. Filter and take filtrate in test tube and add 3-4 drops of 1 percent barium chloride solution. Observe for precipitate formation.

15.12.3 *Interpretation*

White precipitate confirms presence of sulphate, if there is no precipitate, the test is negative.

15.13 Test for Presence of Anionic Detergent in Milk

15.13.0 *General*

Alkyl benzene sulphonic acid (ABS) or anionic detergent may be present in milk due to intentional addition of detergent in milk or due to insufficient rinsing of dairy equipments. The following method is based on the ionic interaction between the anionic detergent and cationic dye. Anionic detergents have a property to form a complex with cationic dyes. The solubility of dye and dye-detergent complex differs significantly as dye-detergent complex is relatively less polar in comparison to dye alone. Formation of dye-detergent complex between cationic dye and anionic detergents and subsequently its extraction into the hydrophobic solvent layer (lower) is the principle behind the method. The method is performed by addition of methylene blue dye solution and chloroform to milk, mixing of the content followed by centrifugation. This results in distribution of dye colour in upper layer and lower layers. Relative intensity of the colour is noticed in these layers. Appearance of relatively more blue colour in lower layer indicates the presence of detergent in milk. The developed test is sensitive to detect anionic detergent up to 0.012 5 percent (12.5 mg/100 ml).

15.13.1 *Reagents*

a) *Methylene blue dye* — 12.5 mg is dissolved in 100 ml of distilled water. Protect the solution against direct sunlight.
b) *Chloroform* (Inflammable and toxic on inhalation. Mouth pipetting is not recommended).

15.13.2 *Procedure*

Pipette 1 ml of suspected milk sample into a 15 ml test tube. Add 1 ml of dye solution followed by addition of 2 ml chloroform. Vortex the contents for about 15 s and centrifuge at about 1 100 rpm for 3 min. Note the intensity of blue colour in lower and upper layer. Relatively, more intense blue color in lower layer indicates presence of detergent in milk. Relatively more intense blue color in upper layer indicates absence of detergent in milk. The method can detect presence of 0.15 percent level of laboratory grade detergent (for example labolene) in milk.

15.14 Detection of Soya Powder in Milk

15.14.0 *General*

Urease enzyme present in soyabean powder converts the urea into carbon dioxide and ammonia. Ammonia dissolves and makes the medium alkaline which turns the colour of phenol red solution to pink.

15.14.1 *Reagent*

15.14.1.1 *Soyabean powder detection solution*

Make 0.1 percent phenol red solution by dissolving in distilled water. Keep it overnight and filter the solution. Dissolve 1 g urea crystals in 100 ml phenol red solution. Keep the solution in cool and dry place. Make fresh solution after one week.

15.14.2 *Procedure*

Take 5 ml of well mixed milk in a test tube. Add 1 ml of soyabean powder detection solution, mix and allow to stand for 10-15 min. Observe the colour. Development of pink colour indicates the presence of soyabean powder in milk.

16 DETECTION OF PRESERVATIVES

16.0 General

Milk and other dairy products are required by Public Health Laws to be free from preservatives. With a few exceptions, any preservative added to milk is not removed in the process of treatment or manufacture. Thus, if milk containing preservatives is accepted, the treated milk or milk product when subsequently offered for sale may contain preservatives and render the seller liable to prosecution besides constituting a hazard to health. No preservative shall, therefore, be added to milk except in the case of the samples which have to be preserved for chemical examination.

Some of the most common preservatives found in milk are boric acid and borax, benzoic acid, formaldehyde and salicylic acid. Hypochlorite residues may also be found in milk when chlorine sterilizers are used for sterilizing milk handling equipment unless such equipment is properly rinsed with water. Antibiotics used in the treatment of udder diseases of milch animals may also find their way in milk if proper precautions are not taken. The presence of chlorine or antibiotics in milk not only enhances the keeping quality of the milk but will reduce the plate counts and also lengthen the M.B.R. (*see* **10**). When testing for preservatives, it is necessary to carry out a control test with a milk sample known to be free from any preservative.

16.1 Test for Boric Acid Using Glycerol

16.1.1 *Reagents*

a) *Phenolphthalein indicator (1 percent)* — Dissolve 1 g of phenolphthalein in 100 ml of 95 percent ethyl alcohol. Add 0.1 N sodium hydroxide solution until one drop gives a faint pink colouration. Dilute with distilled water to 200 ml.
b) *Standard sodium hydroxide solution* — 0.1 N. Prepare a concentrated stock solution of

sodium hydroxide by dissolving equal parts of sodium hydroxide (sticks or pellets) in equal parts of water in a flask. Tightly stopper the flask with a rubber bung and allow any insoluble sodium carbonate to settle out for 3 to 4 days. Use the clear supernatant liquid for preparing the standard 0.1 N solution. About 8 ml of stock solution is required per litre of distilled water. Standardize the prepared 0.1 N solution against standard oxalic acid solution (0.1 N).

c) *Glycerol (50 percent)* — Take 50 ml glycerol and make up to 100 ml with distilled water.

16.1.2 *Procedure*

Take 10 ml milk in a 50 ml beaker. Add 1 ml 1 percent phenolphthalein indicator. Titrate against 0.1 N NaOH solution till pink colour persist for 10-15 s. Add 3-4 drops of 0.1 N NaOH. Milk will be pinking in colour. Divide the sample in two parts in test tubes. In one test tube, add 2.0 ml distilled water and in another test tube, add 2 ml glycerol (50 percent). Compare the colour of glycerol added test tube with the water added test tube. Disappearance of pink) colour or fading of colour in the test tube added with glycerol will indicate the presence of boric acid and borates. The other test tube added with water will remain pink.

16.2 Hehner Test for Formaldehyde

16.2.1 *Reagent*

Concentrated sulphuric acid, commercial; sp gr 1.84.

16.2.2 *Procedure*

To about 10 ml of milk in a wide mouthed test-tube add about half the volume of concentrated sulphuric acid pouring the acid carefully down the side of the tube so that it forms a layer at the bottom without mixing with the milk. A violet, or blue colour, at the junction of the two liquids indicates the presence of formaldehyde. The test is sensitive to one part in 10 000.

NOTE — The test is given only in the presence of a trace of ferric chloride or other oxidizing agents. This test may be combined with the determination of fat (*see* **13**), noting whether a violet colour forms on addition of the sulphuric acid in the butyrometer.

16.3 Test for Benzoic Acid

16.3.1 *Reagents*

a) *Dilute hydrochloric acid,* 1 : 3 by volume.
b) *Ethyl ether*
c) *Ammonium hydroxide,* sp gr 0.88.
d) *Ferric chloride solution,* 0.5 percent (*w*/*v*), neutral.
e) *Sodium hydroxide solution,* 10 percent (*w*/*v*).
f) *Potassium nitrate,* crystals.
g) *Concentrated sulphuric acid,* sp gr 1.84.
h) *Ammonium sulphide,* freshly prepared and colourless.

16.3.2 *Procedure*

Acidify 100 ml of milk with 5 ml of the hydrochloric acid. Shake until curdled. Filter and extract the filtrate with 50 to 100 ml of ether. Wash the ether extract layer with two 5 ml portions of water. Evaporate the greater portion of ether in a porcelain dish on a water-bath and allow the remainder to evaporate spontaneously. If benzoic acid is present in considerable quantity, it will crystallize from the ether in shining leaflets and give a characteristic odour on heating.

Dissolve the residue in hot water, divide into two portions and test as follows:

a) Make one portion alkaline with a few drops of ammonium hydroxide, expel the excess of ammonia by evaporation, dissolve the residue in a few millilitres of hot water, filter if necessary. Then add a few drops of the neutral ferric chloride solution. A salmon coloured precipitate of ferric benzoate indicates the presence of benzoic acid.
b) To the other portion, add one or two drops of sodium hydroxide solution and evaporate to dryness. To the residue, add 5-10 drops of concentrated sulphuric acid and a small crystal of potassium nitrate. Heat for 10 min in a glycerol bath at 120 to 130°C, or for 20 min in a boiling water-bath. The temperature of glycerol bath shall not exceed 130°C. After cooling, add 1 ml of water, make distinctly ammoniacal and boil the solution to decompose any ammonium nitrate that may have been formed. Cool, pour into a test-tube, and add a drop of fresh colourless ammonium sulphide, without allowing layers to mix.
c) A red-brown ring indicates benzoic acid. On mixing, the colour diffuses through the whole liquid, and on heating finally, changes to greenish yellow. This differentiates benzoic acid from salicylic acid. The latter forms coloured compounds, which are not destroyed by heating.

16.4 Ferric Chloride Test for Salicylic Acid

16.4.1 *Reagents*

a) *Dilute hydrochloric acid* — 1 : 3 by volume.
b) *Ethyl ether*
c) *Ferric chloride* — 0.5 percent, neutral.

16.4.2 *Procedure*

Acidify 100 ml of milk with 5 ml of dilute hydrochloric acid. Shake until curdled and filter. Extract with 50 to 100 ml of ether. Wash the ether layer with two 5 ml portions of water. Evaporate the greater portion of ether in a porcelain dish on a steam-bath, allow the remainder to evaporate off. Add one drop of the ferric chloride solution. A violet colour indicates the presence of salicylic acid.

16.5 Test for Presence of Hydrogen Peroxide

16.5.1 *Procedure*

Dissolve 1 g of vanadium pentoxide (V_2O_5) in 100 ml of 6 percent H_2SO_4 (6ml concentrated H_2SO_4 + 94 ml distilled water). To 10 ml of sample add 10-20 drops of reagent and mix.

16.5.2 *Interpretation*

The development of pink or orange red colour indicates presence of H_2O_2 in milk.

16.6 Detection of Hypochlorite

16.6.1 *Apparatus*

- a) *Centrifuge*
- b) *Tubes for centrifuge* — each of capacity 12.5 ml.
- c) *Mercury vapour lamp* — fitted with a Wood's filter.

16.6.2 *Reagent*

16.6.2.1 *Stannous chloride solution* — 0.025 percent (*w/v*) in 73.5 percent sulphuric acid (prepared by mixing three volumes of concentrated sulphuric acid and one volume of water).

16.6.3 *Procedure* — Cool about 3 ml of milk taken in a test-tube in a freezing mixture of ice and salt to 2 to 5°C. In another test-tube, take an equal volume of the stannous chloride solution and similarly cool and add to milk. Shake the tube whilst in the freezing mixture and hold for 3 min. Place the mixture in a 12.5 ml centrifuge tube and centrifuge for 3 minutes at 2 500 rpm. A yellow-green colour is produced in the presence of hypochlorite. Alternatively, after centrifuging, examine the tube in ultra-violet light from a mercury vapour lamp fitted with a Wood's filter, for the presence of any yellow fluorescence.

16.7 Qualitative Detection of Thiocyanates in Milk

16.7.0 *General*

Thiocyanates are added to milk with/without H_2O_2 to increase the keeping quality of milk, but such a practice of addition of thiocyanates to milk is not only against food laws but it can also cause hazardous effect on the health of consumers. The natural levels of thiocyanates in milk are reported to range between 6 to 10 mg/litre with an average of about 8 mg/litre of milk. Thiocyantes can be detected in milk, after deproteinization with trichloroaceitic acid (TCA). TCA filtrate gives orange to orange red color with ferric nitrate in case of added thiocanates, but gives yellow color (with slight orange tinge) due to the thiocyanates naturally present in milk. This test can detect as low as 0.6 mg/litre of added thiocyanate (equivalent to 1 mg/litre potassium thiocyanate).

16.7.1 *Reagents*

- a) *Trichloro acetic acid (TCA),* 20 percent (*w/v*) — Dissolve 20 g TCA in 10 ml of distilled water and filter.
- b) *Nitric acid (HNO_3)*, 2 M — Prepare by diluting 138.5 ml of 65 percent HNO_3 to 1 000 ml with distilled water.
- c) *Ferric nitrate reagent* — Dissolve 16.0 g $Fe(NO_3).9\ H_2O$ in 50 ml of 2 M HNO_3 and dilute to 100 ml with distilled water. Filter through Whatman filter paper No.1 or 4 and store in dark or in amber colored bottle.

16.7.2 *Procedure* — The thiocyanate test in milk can be done in following two ways:

16.7.2.1 *Test applicable to TCA filtrate* — Take 6 ml of milk sample and add 3 ml TCA (20 percent) to precipitate the proteins. Filter through Whatman no. 40 filter paper. To a clear 1.5 ml filtrate, add 1.5 ml of Ferric nitrate reagent. Run the black simultaneously using distilled water instead of milk. Observe the intensity of colour in case of blank and milk sample and compare with the Table 1 to detect the presence of extraneous thiocyanate in milk.

Table 1 The Order of Colour Intensity for Detection of Thiocyanate in Milk (Test Applicable to TCA Filtrate and Directly to Milk)

(*Clause* 16.7.2.1)

Sl No.	Sample	Level of Thiocyanate Added (mg/litre)	Hue and Intensity of Colour
i)	Blank	—	Transparent
ii)	Control (milk)	—	Yellow (with slight orange tinge)
iii)	Test sample1)	1	Yellowish-orange
iv)	Test sample1)	2-4	Orangish-red
v)	Test sample1)	>5	Red

1)Test sample contains milk and added thiocyanate.

16.7.2.2 *Test applicable directly to milk* — Take 5 ml of milk sample and add equal quantity of Ferric nitrate reagent (5 ml). For blank, take distilled water instead of milk. Observe the colour in both the cases. For further clarity, filter the milk sample after developing colour through Whatman no. 40 filter paper. Observe the intensity of colour in case of blank and milk sample and compare with Table 1 to detect the presence of extraneous thiocyanate in milk.

17 DETECTION OF NEUTRALIZERS

17.0 General

Neutralizers in the form of lime water, or sodium bicarbonate may be added to neutralize developed acidity before milk is processed. Such a practice is not permissible.

17.1 Rosalic Acid Test for Carbonates

17.1.1 *Reagents*

Rosalic acid solution (0.05 percent rosalic acid) in 60 percent ethyl alcohol solution (60 : 40 ethyl alcohol and water).

NOTE — The alcohol used for preparation of 60 percent (*v/v*) alcohol is to be distilled or absolute alcohol.

17.1.2 *Procedure*

Take 2 ml rosalic acid solution (0.05 percent rosalic acid) in a test tube and add 2 ml of milk.

17.1.3 *Interpretation*

Rose-red colour development indicates neutralizer presence in milk.

17.2 Detection of Added Sodium Salts

17.2.0 *General*

Milk has natural sodium as mineral in milk serum. However, in case of added sodium in the form of alkaline water or sodium salt, it will be reflected in higher sodium ions which can be tested with sodium detection meters.

17.2.1 *Apparatus*

A potentiometeric titrator/ISE meter Hach or Metler Toledo or any other manufacturing companies with sodium electrodes DX-223 and reference electrodes DX-200 in case of instrument No. DL-50 (2.4 version).

17.2.2 *Reagents*

17.2.2.1 *Preparation of ion strength adjuster (I.S.A.) buffer*

100 g NH_4CI in 250 ml of deionised water + 25 ml of NH_4OH (Ammonia solution) and make up the volume to 500 ml in volumetric flask.

17.2.2.2 *Preparation of sodium standard solution (1 000 mg/litre)*

Take 1.2706 g of dried NaCl (dried in oven at 120°C for 3 to 4 hours) make the volume to 500 ml in a volumetric flask.

17.2.2.3 *Preparation of sodium standard solution (100 mg/litre)*

Take 50 ml of sodium solution (1000 mg/litre) prepared as above and make up the volume to 500 ml mark.

NOTE — Storage of (a) Na^+ electrode in 0.1 M NaCl solution, and (b) Reference electrode in I.S.A. solution.

17.2.3 *Calibration Done at Method I.D. – 104*

a) Stage calibration with 1000 mg/litre [Na^+]
b) Stage calibration with 100 mg/litre [Na^+]

17.2.4 *Procedure*

Take 10 ml of milk, dilute to 100 ml with 90 ml distilled water. Add 2 ml of ISA buffer and proceed for estimation of sodium ions by inserting sodium electrodes and reference electrodes in the sample.

17.2.5 *Interpretation*

The normal range of sodium ions in the raw fresh milk is between 350 to 600 ppm, if the content is higher than the normal, it shows added sodium.

17.3 Acidity Difference Before and After Boiling the Sample for Neutralizer Detection

17.3.0 *General*

Milk is heterogeneous mixture of colloidal particles, water, minerals, vitamins and fat soluble constituents. It has also absorbed carbon dioxide and other volatile compounds which get evolved thereby lowering the acidity and increasing the *p*H slightly. In case of normal milk, the difference in acidity before and after boiling is upto 0.02 percent and *p*H also differs by 0.02 unit. If the milk is neutralized or stabilized, the acidity difference increases from 0.02 to 0.03 and more. The difference between before and after boiling acidity should not be more than 0.02 percent.

17.3.1 *Reagents*

0.1 N NaOH, Rosalic acid solution.

17.3.2 *Procedure*

Take 10 ml of milk and determine acidity as per the normal procedure (*see* **12**). Boil another 10 ml aliquot of the milk sample for one to two minutes. Cool to room temp and determine acidity again. If the difference between normal and after boiling acidity is more than 0.02 percent the milk can be doubted for neutralization and it will correlate with poor taste, slightly high *p*H and also in the rosalic acid colour.

18 DETECTION OF MASTITIS

18.0 General

Mastitis or inflammation of the udder is caused by certain species of streptococci, principally

Streptococcus agalactia, staphylococci and a few other types of organisms. Though in most cases mastitis is caused by the non-pathogenic types of bacteria, their occurrence interferes with the efficient secretion of milk and is invariably a source of great economic loss. It is, therefore, necessary to regularly examine all the milking animals in a herd to ensure that none is suffering from mastitis, and whenever positive cases are detected, to take prompt measures to check and eliminate the disease.

It is essential that the test for the detection of mastitis be made on single quarter samples since milk from normal quarters may obscure detection of the abnormal ones if composite samples are taken. Any quarter giving an abnormal reaction should then be followed up. The first 2 or 3 streams of milk shall always be used for diagnostic tests as these will show the greatest change if the quarter is abnormal.

18.1 Bromothymol Blue Test

This test is based on the fact that milk from infected udders is usually alkaline and shows *p*H as high as 7.4. The indicator solution may be prepared by triturating 1 g of bromothymol blue powder with 160 ml of exactly 0.01 N sodium hydroxide solution, and diluting with 590 ml of distilled water and the test carried out as follows:

> Place 1 ml of bromothymol blue solution in a test-tube and add 5 ml of milk. Mix and observe the colour (*see* **7**).

18.2 Direct Microscopic Somatic Cell Count

18.2.0 *General*

Bacterial infection causes mastitis or inflammation of the mammary glands. Somatic cell counts have animal health, milk quality and productivity implication. Mastitis causes diminished production of milk due to tissue damage of the glands. The concentration of the somatic cells in the milk is directly related to infection status of udder. If the cell count is high, the milch animal has mastitis or inflammation of the mammary glands. When the bacterial infection starts in the mammary glands, the milch animals evolve complex internal body system that enables them to defend and get rid themselves of disease causing bacteria. They have the ability to mobilize and direct bacteria fighting cells to the udder. These cells are termed 'somatic cells or leucocytes' which move to the infection site to minimize the infection by surrounding or internalizing the bacteria. Finally the somatic cells help eliminate infection. If the somatic cell count is more than 5×10^5, it is an indication that the milch animal is infected. Therefore, in the day-to-day management of dairy, infection status of the milch animal can be controlled effectively by monitoring the somatic cell count of bulk milk or individual milch animal.

18.2.1 *Principle*

Stained films of a measured volume of milk spread on glass slides of specified area is examined under a Microscope. The microscope is first calibrated so that the exact area of the Microscopic field is known. Then a measured quantity of milk (0.01 ml) is spread over a measured area of one square cm on a cleaned glass slide so that each microscopic field examined represents a quantitative aliquot of the sample. The milk (0.01ml) is allowed to dry, and the film is defatted, fixed and stained with a suitable dye such as Newman's stain.

The average number of somatic cells/microscopic field is determined after examining between 5 and 60 fields, depending upon the microscopic factor and the number of somatic cells/field. This method requires 10-15 min for examining one sample. Direct microscopic method makes it possible for milk to be examined for numbers of somatic cells.

The number of somatic cells in raw milk provides measure of the extent of mastitis. Stained somatic cells are visible under the Microscope and can be taken as way of assessing raw milk quality.

18.2.2 *Apparatus*

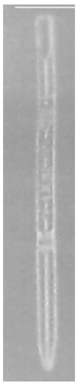
18.2.2.1 *Microscope* — Having 1.8 mm oil immersion objective.

18.2.2.2 *Microscopic slides* — Size 2.5 cm × 7.5 cm.

They may be either plane or with clear, circular 1 cm^2 areas (diameter approx. 11.28 mm) or a square of 1 cm^2 areas demarcated on the slides. New slides should be cleaned and dry. The slides maybe cleaned by soaking in cleaning solution of chromic acid (prepared by dissolving 50 g of sodium dichromate in 200 ml of water in a glass or earthen container and then adding carefully 300 ml of concentrated sulphuric acid of commercial grade), rinsing in flowing tap water and finally in distilled water. Used slides should be socked in hot or boiling alkaline detergent solution until all residues are removed, then rinse in flowing tap water, dried and properly stored for re-use.

18.2.2.3 *Micropipette or micro syringe for transfer of 0.01 ml of milk* — Breed's pipettes which delivers 0.01 ml or micro syringe delivering 0.01 ml. After use these pipettes should be thoroughly cleaned with cleaning solution and rinsed with clean water.

0.1 ml Syringe



Breed's pipette

18.2.2.4 *Guide slide*

Glass or cardboard Guides ruled into round or square areas each one of a square cm inscribed thereon, for use beneath clean slide so that the milk may spread over a space of exactly 1 cm^2. The slides having a square area engraved on the slides are also available.

18.2.2.5 *Needle, bent point*

Suitable for spreading milk over an area of 1 cm^2.

18.2.2.6 *Drying device*

Level surface at 40-45°C or cabinet over electric bulb for drying the slides.

18.2.2.7 *Forceps or slide holders*

Suitable for dipping and holding slides.

18.2.2.8 *Jars or beakers*

Of suitable size for staining by submersing the slides in the Newman's stain poured in the jars or beakers.

18.2.2.9 *Stage micrometer*

Having fine graduation measuring in 1/100th mm (0.01 mm).

18.2.3 *Reagents*

18.2.3.1 *Newman's stain*

18.2.3.1.1 Reagents for preparation of Newman's stain:

a)	Methylene blue chloride	1 g
b)	Glacial acid	6 ml
c)	Methyl Alcohol	54 ml
d)	Tetra chloroethne	40 ml

18.2.3.1.2 *Preparation of Newman stain*

Add the alcohol to tetrachloroethane and heat to 70°C in a water-bath and add this warm mixture to methylene blue powder and shake vigorously until the dye is completely dissolved. Cool and then slowly add acetic acid and mixed. Filter the mixture and store in a tightly stopper bottle. The purpose of this stain is three fold : to fix the film of the milk on the glass slide, to dissolve the fat and to stain the somatic cells.

18.2.4 *Estimation of Microscopic Factor*

First of all, determine the diameter of the field, using a stage micrometer. The micrometer is a slide having fine graduation measuring in 1/100th mm.

Suppose the diameter of a field is 0.16 mm, the area of the field is

$$\pi r^{2} = 3.141\,6 \times (0.08)^2$$
$$= 3.141\,6 \times 0.006\,4$$
$$= 0.02 \text{ mm}^2 \text{ approx.}$$

where 0.08 is the radius of the field.

The next step is to determine the number of fields in the entire film to be examined. The size of this film is 0.01ml spread over 1 cm^2 or 100 mm^2

Therefore, number of fields = 100/0.02

$$= 100 \times 100/2$$
$$= 5\,000$$

This figure represents 0.01 ml of milk.

The figure for 1 ml of milk would be = 5 000 × 100 = 500 000

This is the microscopic factor.

The average number of somatic cells counted in several fields must be multiplied by this factor to get the number of somatic cells in one ml. of milk. Thus, if 50 fields are examined and the average number of the somatic cells found is 0.6, the somatic cell number per ml. will be 500 000 × 0.6 = 300 000.

18.2.5 *Procedure*

18.2.5.1 *Preparation of milk film on microscopic slides*

Take the well mixed sample of milk using Breed's pipette or micro syringe and discharge the entire volume of 0.01 ml of milk on to the center of the one cm square area of the slide. Spread the milk uniformly over the entire one cm^2 area and dry the film at level surface at 40-45°C in warm atmosphere or on cabinet over electric bulb as shown in Fig. 5 to Fig. 8.

18.2.5.2 *Defatting, fixing and staining of the smear*

Dip the slides in Newman's stain for 1/2 to 1 minute. Remove the slides and drain off the excess stain and allow to dry thoroughly. Rinse the slides in three exchanges of water and then drain off excess water and air dry before examining the film under microscope as shown in Fig. 9 to Fig. 14.

18.2.5.3 *Examination of stained film*

Examine the film with an oil immersion objective after placing one drop of immersion oil on the film. The fields for counting should be selected at random and represent all parts of the film. The following guide can

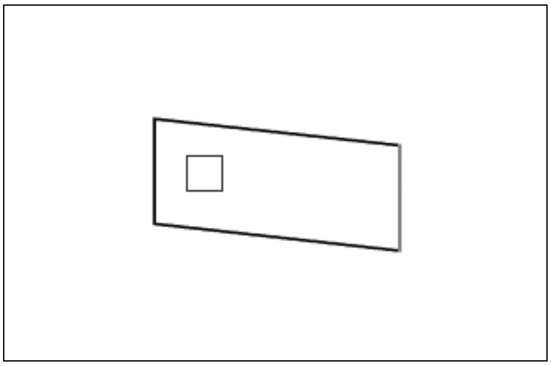


FIG. 5 GUIDE SLIDE RULED INTO cm^2 AREA AND TO BE PLACED UNDER THE SLIDE FOR MAPPING ONE cm ^{2} AREA FOR PREPARING SMEAR

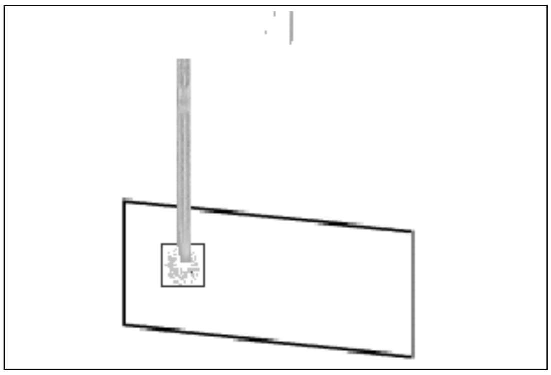


FIG. 6 PLACE 0.01 ml MILK AT THE CENTER OF THE SQUARE BY PLACING THE SLIDE OVER THE GUIDE

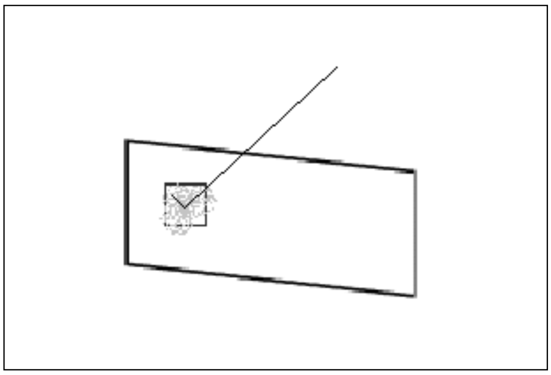


FIG. 7 SPREAD THE MILK UNIFORMLY WITH THE HELP OF A WIRE SLIGHTLY TURNED AT THE END AND DRY IT IN WARM ATMOSPHERE OR ON LEVEL SURFACE AT 40-45°C OR OVER THE CABINET

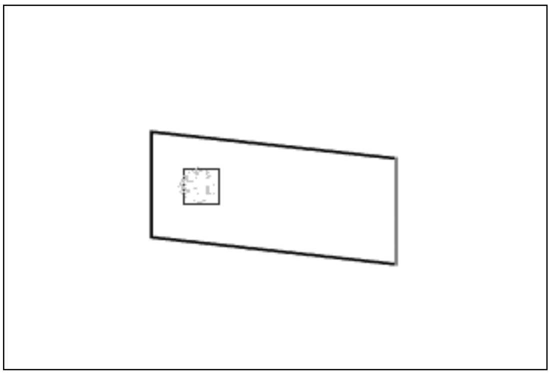


FIG. 8 DRY SMEAR FOR STAINING

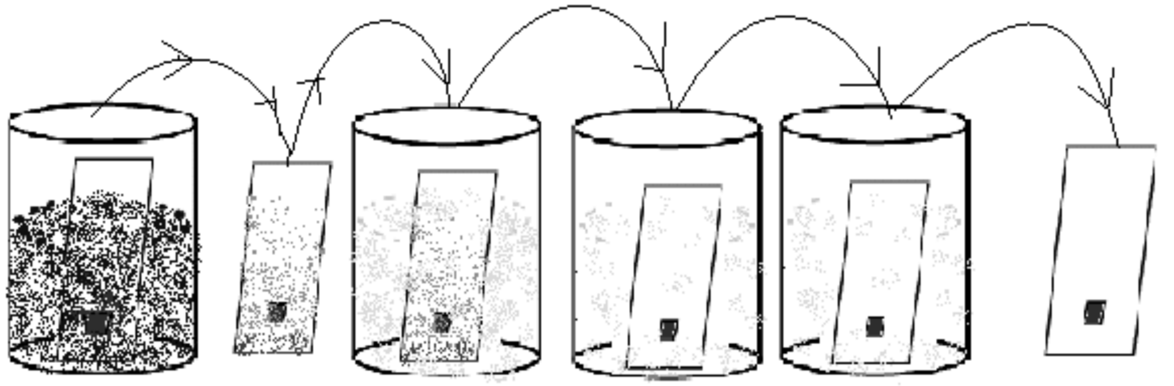


FIG. 9 DIP THE SLIDE IN NEWMAN'S STAIN

FIG. 10 REMOVE THE EXCESS STAIN AND DRY

FIG. 11 RINSE THE SLIDE BY PLACING IT IN WATER FOR ONE MINUTE TO REMOVE THE EXCESS DYE

FIG. 12 FIG. 13 SIMILARLY RINSE THE SLIDE BY PLACING IT IN WATER FOR ONE MINUTE IN TURN

FIG. 14 AIR DRY THE SLIDE AND EXAMINE UNDER MICROSCOPE

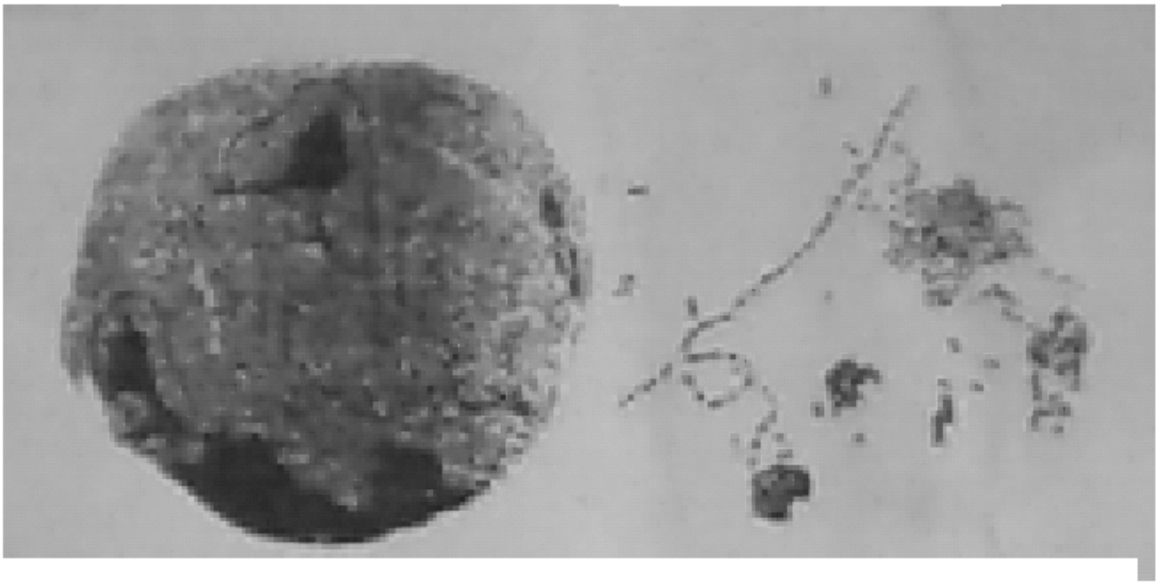


FIG. 15 SOMATIC CELLS PLUS LONG CHAIN OF BACTERIA INDICATING MASTITIS

be used for counting number of fields to be examined.

Average number of cells	*Number of fields to be examined*
<0.05	50
0.5-1.0	25
1-10	10
10-30	5

Count only somatic cells with an identifiable stained nucleus. For polymorph nucleated cells, count as a single cell if it has two or more discernible nucleus lobes. For other somatic cells, count the ones that have a nucleus which appears to be essentially intact. A large number of somatic cells plus long chain of bacteria ten or more organism in a chain indicate mastitis as shown in Fig. 15.

Mastitic milk comes from a milch animal shedding *Streptococcus agalactiae*. These long chain streptococci and associated leucocytes are frequently seen in mastitic milk. The long chains of *S. agalactiae* plus large numbers of leucocytes are typical of mastitis (*see* Fig. 16). However, this is only one of the many organisms found in milk from milch animal with damage udder condition.

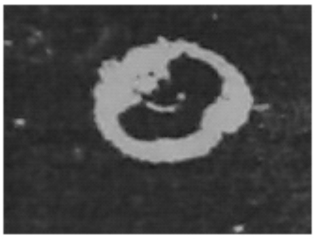


FIG. 16 APPEARANCE OF STAINED LEUCOCYTES

18.2.6 *Interpretation*

The somatic cell count more than 5×10^5 is an indication that milch animal is infected.

19 HANSA TEST

19.0 Hansa test is used to detect the adulteration of cow milk with buffalo milk.

19.1 Instructions for Preservation of Sample

19.1.1 As soon as the serum is received, transfer it to a refrigerator or ice box. It should be preserved at a temperature of 4°C or as near to this temperature as possible. But it should not be frozen. Remember that it is a living tissue and its life depends on the care and gentleness with which it is handled. It should always be thoroughly mixed before use, but not shaken in such a way that foam appears.

19.2 Cleanliness

19.2.1 Keep all glassware and polytheneware thoroughly clean. The slides and pipettes should be thoroughly washed in distilled water and spirit, and dried before and after use each time. Be most careful about the pipettes introduced into the serum, which should be absolutely clean and dry. With care it is possible to keep the equipment clean even under the most primitive village conditions, but without cleanliness it is not possible to get good results.

19.3 Procedure

19.3.1 Place 2 to 3 ml (accurately measured) of the milk sample to be tested in a test-tube. Label it according to a convenient code. Add about 19 times the quantity of water to the milk, so that the sample is diluted 1/20 with water. Take a pipette fitted with a rubber bulb. Put a drop of the mixture on the centre of a glass slide. With another pipette, take out a drop of serum after proper mixing. Place a drop of the serum on the drop of milk (without touching the milk with the pipette). Mix the two-drops together thoroughly with a clean tooth pick or glass rod. Pick up the slide and gently rock it in your hand so as to give the whole material a swirling motion. Curdy particles develop in the milk containing buffalo milk within half minute. If such particles do not develop for half a minute, the test is negative and there is no buffalo milk present in the sample (provided the serum is in good condition and the test was carried out properly).

19.3.2 *Control*

Before testing unknown samples, test several known samples of pure cow milk, buffalo milk, and cow milk containing buffalo milk in the minimum possible proportion (say 10 percent). Also, it would be a good idea to take another drop of diluted milk and test it with a drop of water in place of serum. If flocculations or particles are observed, the milk sample is spoilt (possibly due to excessive acidity) and is unsuitable for the test by the standard technique.

19.4 Preserved Milk Sample

Milk preserved with any of the standard preservatives and kept in the refrigerator can be used for Hansa test for as long as two weeks. Most satisfactory results have been obtained with 0.4 percent formalin (mix drops of commercial formalin in 28.4 ml). Boiling, addition of sugar and other common additives and stabilizers have no effect on the test.

19.5 Life of the Serum

If all the precautions are observed, and the serum is always handled with care, it should be possible to use it up to three months. In old sera, precipitates may sometimes be found, and these may cause errors in reading the test. The particles can be seen when sera are taken in a pipette. They can be easily removed by centrifugation.

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